

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
8 January 2004 (08.01.2004)

PCT

(10) International Publication Number
WO 2004/003232 A1

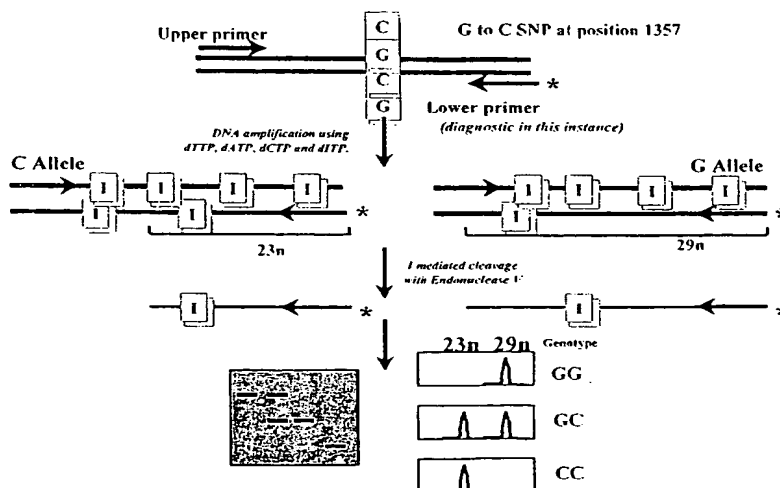
- (51) International Patent Classification⁷: C12Q 1/68 (74) Agent: ANNE RYAN & CO; 60 Northumberland Road, Ballsbridge, Dublin 4 (IE).
- (21) International Application Number: PCT/IE2003/000098 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 25 June 2003 (25.06.2003) (25) Filing Language: English (26) Publication Language: English
- (30) Priority Data: 2002/0544 28 June 2002 (28.06.2002) IE (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicants (*for all designated States except US*): HIBER-GEN GENOMICS LIMITED [IE/IE]; IDA Business Park, Southern Cross Road, Bray, County Wicklow (IE). UNIVERSITY COLLEGE CORK-NATIONAL UNIVERSITY OF IRELAND, CORK [IE/IE]; College Road, Cork (IE).
- (72) Inventors; and (75) Inventors/Applicants (*for US only*): VAUGHAN, Patrick, Martin [IE/IE]; 7 Glenageary Lodge, Glenageary, County Dublin (IE). MCCARTHY, Thomas, Valentine [IE/IE]; Montenotte, Cork (IE).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE CHARACTERISATION OF NUCLEIC ACID MOLECULES



(57) Abstract: A method for characterising nucleic acid molecules comprises the steps of i) introducing a modified base which is a substrate for a DNA endonuclease, for example Endonuclease V from *E. coli*, into a target DNA molecule; and ii) reacting the nucleic acid containing the modified base with said DNA endonuclease such that the nucleic acid is cleaved to generate an upstream fragment condition said modified base and bearing a 3' hydroxyl group. The upstream fragment generated in step ii) can be used as a primer for a subsequent extension reaction. The modified base as such targets cleavage of the nucleic acid strand because it directs the endonuclease to cut the nucleic acid strand on which it resides at a site predominantly one nucleotide removed therefrom. so that the modified base remains in the nucleic acid as the upstream fragment. The method can be used for detecting polymorphisms and mutations and for nucleic acid identification and profiling. The method enables one to detect polymorphisms in any sequence context and such detection is not dependent on surrounding nucleotide sequence.